Effect of 4-Aminopyridine on Genioglossus Muscle Activity during Sleep in Healthy Adults

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Abstract

Rationale: The reduction in upper airway muscle activity from wakefulness to sleep plays a key role in the development of obstructive sleep apnea. Potassium (K^+) channels have been recently identified as the downstream mechanisms through which hypoglossal motoneuron membrane excitability is reduced both in non-rapid eye movement (NREM) sleep and REM sleep. In animal models, the administration of 4-aminopyridine (4-AP), a voltage-gated K^+ channel blocker, increased genioglossus activity during wakefulness and across all sleep stages.

Objectives: We tested the hypothesis that administration of a single dose of 4-AP 10 mg extended release would increase genioglossus activity (electromyography of the genioglossus muscle [EMG_{GG}]) during wakefulness and sleep, and thereby decrease pharyngeal collapsibility.

Methods: We performed a randomized controlled crossover proof-of-concept trial in 10 healthy participants. Participants received active treatment or placebo in randomized order 3 hours before bedtime in the physiology laboratory.

Results: EMG_{GG} during wakefulness and NREM sleep and upper airway collapsibility measured during NREM sleep were unchanged between placebo and 4-AP nights. Tonic but not phasic EMG_{GG} during REM sleep was higher on the 4-AP night when measured as a percentage of maximal voluntary activation (median [interquartile range] 0.3 [0.5] on placebo vs. 0.8 [1.9] $\%_{max}$ on 4 AP; $P = 0.04$), but not when measured in μV or as a percentage of wakefulness value.

Conclusions: A single dose of 4-AP 10 mg extended release showed only a small increase in tonic EMG_{GG} during REM sleep in this group of healthy subjects. We speculate that a higher dose of 4-AP may further increase EMG_{GG}. However, given the potentially severe, dose-related adverse effects of this drug, including seizures, the administration of 4-AP does not appear to be an effective strategy to increase genioglossus activity during sleep in humans.

Clinical Trial registered with clinicaltrials.gov (NCT02656160).

Keywords: obstructive sleep apnea; potassium channel blockers; drug therapy

(Received in original form January 3, 2017; accepted in final form March 24, 2017)

This research project received generous philanthropic funding from Fan Hongbing, president of the OMPA Corporation, Kaifeng, China. This work was also supported by National Institutes of Health Grants R01 HL102321 and P01 NIH HL095491, as well as Harvard Catalyst Clinical Research Center Grant UL1 RR 025758–01. L.T.-M. is supported by the American Heart Association (17POST33410436). S.A.S. is supported by the American Heart Association (15SDG25890059), the National Health and Medical Research Council (Early Career Fellowship 1,053,201), the Menzies Foundation, and the American Thoracic Society Foundation, and was coinvestigator on grants from the National Health and Medical Research Council (1,064,163) and the National Institutes of Health (R01 HL128658). M.M. is supported by the São Paulo Research Foundation. B.A.E. was supported by the National Health and Medical Research Council of Australia's CJ Martin Overseas Biomedical Fellowship (1,035,115) and is now supported by a Heart Foundation of Australia Future Leader Fellowship (101,167). D.J.E. is supported by a National Health and Medical Research Council Senior Research Fellowship (1,116,942).

Author Contributions: L.T.-M. contributed to study design, data collection, data analysis and interpretation, and drafting and review of the manuscript for important intellectual content; S.A.S. contributed to study design, data collection, data analysis and interpretation, and review of the manuscript; A.A., M.M., and C.M.d.M. contributed to the data collection and review of the manuscript; B.A.E, D.J.E., L.M., and D.P.W. contributed to data interpretation and review of the manuscript for important intellectual content; and A.W. contributed to the study design, data analysis and interpretation, and drafting and review of the manuscript for important intellectual content.

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Ann Am Thorac Soc Vol 14, No 7, pp 1177–1183, Jul 2017

DOI: 10.1513/AnnalsATS.201701-006OC
Internet address: www.atsjournals.org

Animal (1, 2) and human (3) research suggests the withdrawal of norepinephrine, serotonin, and other monoamines at the hypoglossal motornucleus contribute to the reduction of upper airway muscle activity from wake to non-rapid eye movement (NREM) sleep. Acetylcholine-induced inhibition instead seems to play a primary role in upper airway muscle atonia during REM sleep through muscarinic M2 receptors (4).

The inhibition or activation of potassium (K^+) channels has more recently been identified as the downstream mechanism through which these monoamines modify the motoneuron membrane excitability both in NREM and REM sleep (5). Thus, it may be an ideal target for pharmacologic treatment of obstructive sleep apnea. Opening of K^+ channels produces a hyperpolarization of neurons, thereby reducing cell excitability (6). It has been shown that REM sleep-mediated atonia of the genioglossus muscle is largely dependent on the activation of a muscarinic receptor associated with a G-protein-coupled inwardly rectifying K^+ channel (4, 7). Two-pore domain acid-sensitive K^+ channels may instead play a role in hypoglossal motoneuron excitability during NREM sleep because they are inhibited by G-protein-coupled receptors of wake-promoting monoamines (6), whose concentration in the central nervous system is reduced from wakefulness to NREM sleep.

To confirm this hypothesis, it has recently been shown that in rats, injection of 4-aminopyridine (4-AP) into the hypoglossal motor nucleus causes an increase in genioglossus activity across all states (wakefulness, NREM, and REM sleep). 4-AP is a selective blocker of fast voltage-gated K^+ channels in excitable tissues and nonexcitable cells such as B and T lymphocytes. The use of 4-AP in its extended-release formulation, at the dose of 10 mg twice a day, is approved in humans affected by multiple sclerosis to increase walking ability (8).

In this study, we tested the hypothesis that administration of 4-AP extended release (Ampyra 10 mg; Acorda Therapeutics, Ardsley, NY) before sleep in healthy individuals would increase genioglossus activity during wakefulness, NREM, and REM sleep compared with placebo. The primary outcome for this study was electromyography of the genioglossus muscle (EMG_{GG}) during wakefulness and sleep. EMG_{GG} values were expressed in microvolts (μV), as a percentage of maximum activation during wakefulness ($\%_{max}$), and as a percentage of baseline quiet wakefulness ($\%_{wake}$). As a secondary outcome, we also assessed the effect of 4-AP on genioglossus muscle responsiveness to negative pressure and pharyngeal collapsibility during sleep.

Methods

Participants

Healthy participants aged 21–65 years were included in the study protocol. Individuals were excluded if they had a clinically diagnosed sleep disorder, were taking medications known to influence breathing or muscle physiology, or had allergies to lidocaine, oxymetazoline-HCl, or 4-AP. Participants with a history of epilepsy or renal impairment were also excluded, as this drug may increase the risk for seizure. The protocol was approved by the Partners Institutional Review Board at Brigham and Women's Hospital (protocol #2014P001033). All subjects provided written informed consent before enrolment in the study.

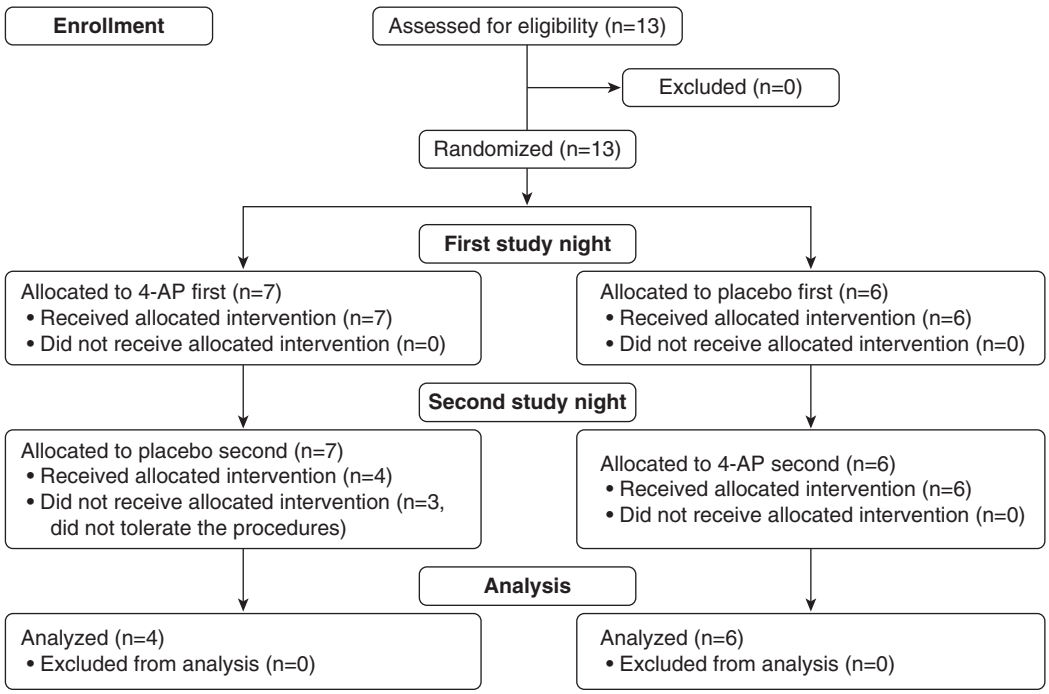

Figure 1. Flowchart of the trial. 4-AP = 4-aminopyridine.

Measurements and Equipment

Anthropometric data were collected on both study nights. In addition to the standard clinical polysomnography montage, participants breathed through a sealed nasal mask attached to a pneumotachometer (Hans-Rudolph, Kansas City, MO) connected to a pressure transducer (Validyne, Northridge, CA). Mask pressure was monitored with a second pressure transducer (Validyne) referenced to atmosphere.

Epiglottic pressure was determined with a small, flexible, pressure-tipped catheter (Millar Instruments, Houston, TX) that was inserted through a decongested (oxymetazoline-HCL) and anesthetized (4% lidocaine) nostril until the tip of the catheter was located 1–2 cm caudal to the base of the tongue.

EMG activity from the genioglossus (EMG_{GG}) muscle was recorded and quantified as described in our previous studies (3, 9–11).

Protocol

Two overnight sleep studies were performed 7 ± 3 days apart: a placebo night and a 4-AP night, with a single-blinded (investigators), randomized control design (see Figure 1 for the flow chart). Randomization was performed by the investigational pharmacy; all data analysis and subject exclusions were performed before unblinding of the intervention allocation. For each night, the subjects arrived at the sleep laboratory at approximately 6:30 P.M. After the measurement of baseline EMG_{GG} activity during wakefulness for 10 minutes, the placebo or 4-AP was administered approximately 3 hours before lights out. Once the patient had been set up for overnight monitoring, the measurements outlined here were performed.

Table 1. Characteristics of the patients analyzed (N=10)

Characteristic	
Female sex, n (%)	6 (60)
Age, yr	26.0 [10]
Body mass index, kg/m^2	24.5 [7.4]
Neck circumference, cm	38.0 [8.3]
Waist circumference, cm	94.5 [22.0]

Data are presented as median [interquartile range] unless otherwise specified.

Table 2. Activity of genioglossus muscle during sleep

	Placebo	4-AP	*P* value
NREM tonic			
μV	10.8 [11.0]	7.5 [18.2]	0.49
%max	0.8 [1.1]	0.7 [1]	0.38
%wake	167 [185]	136 [354]	>0.5
NREM phasic			
μV	9.4 [14.3]	5.4 [26.7]	0.38
%max	0.5 [1.5]	0.6 [1.5]	0.28
%wake	245 [228]	163 [500]	0.32
REM tonic			
μV	3.9 [6.5]	10.6 [20.8]	>0.5
%max	0.3 [0.5]	0.8 [1.9]	0.04
%wake	83 [80]	92 [212]	>0.5
REM phasic			
μV	5.0 [16.3]	0.8 [12.1]	0.46
%max	0.5 [0.9]	0.4 [3.6]	0.38
%wake	170 [523]	144 [387]	>0.5

Definition of abbreviations: 4-AP = 4-aminopyridine; NREM = non-rapid eye movement sleep; REM = rapid eye movement sleep.
Data are expressed as median [interquartile range]. REM values reflect both active periods characterized by sporadic muscle twitches and passive periods characterized by muscle atonia.

Wake-vs.-Sleep EMG_{GG} at Atmospheric Pressure

At least 10 minutes of quiet wakefulness was recorded to quantify each subject's awake EMG_{GG} activity in the lateral position. We chose the lateral position to minimize airway narrowing and pharyngeal pressure swings that reflexively alter genioglossus activity (3, 12). At least 1 hour of sleep data were then collected during NREM and REM sleep in the lateral position before upper airway physiology was assessed.

Upper Airway Physiology Using Continuous Positive Airway Pressure Manipulation

Participants were placed supine and connected to a positive/negative pressure source (Philips-Respironics, Murrysville, PA) to enable rapid switching between pressure levels. When stable sleep was reached, the pressure in the mask was increased to the required level to abolish flow limitation, as determined by the airflow waveform and epiglottic pressure signals. After a baseline recording period of 5 minutes, the continuous positive airway pressure (CPAP) level was reduced to varying suboptimal pressures using two approaches. In the first approach, CPAP was lowered gradually (<1 cm H_2O/min) to slowly reduce ventilation and thereby increase epiglottic pressure swings and pharyngeal muscle activity. During this procedure, we assessed the EMG_{GG} response to progressively greater negative epiglottic pressure swings (genioglossus muscle responsiveness). In the second, CPAP was lowered acutely to subtherapeutic levels to assess the upper airway collapsibility under eupneic (passive) conditions. See *Data Analysis* for further details. After approximately 2 hours of sleep under these conditions, CPAP was removed and participants returned to the lateral position to collect additional EMG_{GG} data at atmospheric pressure.

Data Analysis

The raw EMG_{GG} was processed and quantified in microvolts (μV) as a percentage of maximal voluntary activation ($\%_{max}$), as previously described (13), and as percentage of quiet wakefulness ($\%_{wake}$) for between-night comparison of baseline sleep EMG_{GG} activity. The patient was asked to perform several maneuvers to determine maximum genioglossus activity, including swallowing and maximally pushing the tongue against the front upper or lower teeth.

EMG_{GG} analysis was performed on a breath-by-breath basis identifying a maximum and a minimum value during inspiration and expiration, respectively (EMG_{GG} peak and tonic). The difference between peak and tonic values was used to estimate the respiratory-related phasic activity.

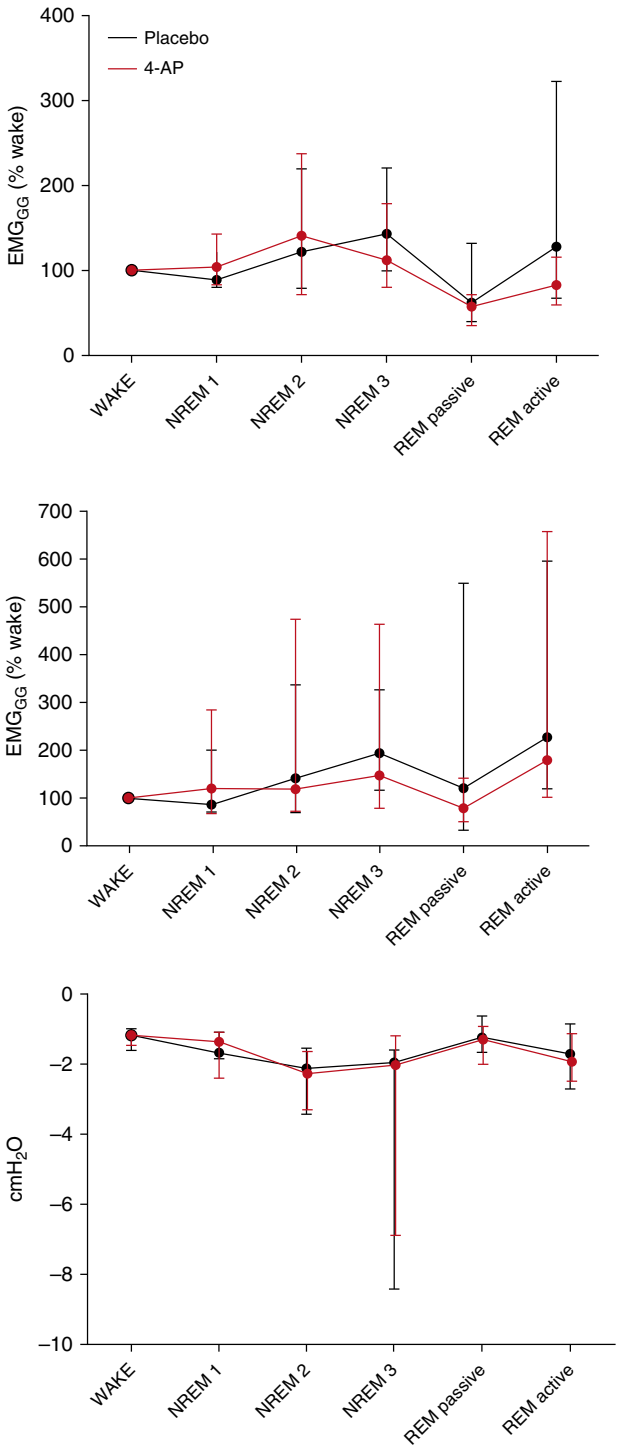

Figure 2. Genioglossus activity and epiglottic pressure across sleep stages during placebo and 4-aminopyridine (4-AP) nights. Group data representing tonic (*upper*), phasic (*middle*) genioglossus activity (EMG_{GG}), and epiglottic pressure swings (*lower*). There was no significant difference between placebo and 4-AP in EMG_{GG} for any sleep stage analyzed when measured as a percentage of wakefulness value. The EMG_{GG} was matched for the same range of epiglottic pressure swings (median value during placebo night ± 1 cm H_2O). *Dots* indicate median values, and *lines* indicate 25th (low) and 75th (top) percentiles. Rapid eye movement (REM) passive refers to periods of relative atonia during REM sleep, whereas REM active refers to periods characterized by prolonged muscle twitches. NREM1, NREM2, and NREM3 = non-rapid eye movement sleep stages 1, 2, and 3.

Wakefulness EMG_{GG} values were obtained from a minimum of 10 epochs (30 seconds each) from the subject lying in the lateral position at baseline and after approximately 3 hours from drug administration.

EMG_{GG} values during sleep were matched for the same epiglottic pressures ranges between nights during NREM and REM sleep.

Apneas and hypopneas were scored using standard American Academy of Sleep Medicine guidelines (14), and the arousal and apnea–hypopnea indices reported are the value taken while the participants slept without CPAP.

As described in previous studies (13, 15), for each participant, we also measured the genioglossus responsiveness to negative pressure (slope of EMG_{GG}) and the passive upper airway collapsibility.

Statistical Analysis

Variables were compared using Wilcoxon matched-pairs signed rank test, with $P < 0.05$ considered statistically significant. Data are expressed as a median (interquartile range). Statistical analyses were performed using Prism 6.0 (Graph Pad Software, La Jolla, CA).

Results

Participants

A total of 13 patients were enrolled in the study. Three patients did not tolerate the study setup, and for this reason, they did not come back for the second study night. Therefore, data from 10 participants were analyzed for upper airway physiology on both nights. The characteristics of these patients are described in Table 1. One patient experienced a mild headache 2 hours after 4-AP administration. The symptom spontaneously disappeared 1 hour later.

Effect of 4-AP on EMG_{GG} Activity during Wakefulness

The number of breaths analyzed during wakefulness before placebo/drug administration was 111 ± 37 during placebo night and 93 ± 43 during 4-AP night. Three hours after placebo/drug administration, 151 ± 98 breaths were analyzed on the placebo night versus 114 ± 90 breaths on the 4-AP night. Maximum voluntary EMG_{GG} was similar between nights (701 [332] on placebo vs. 625 [414] μV

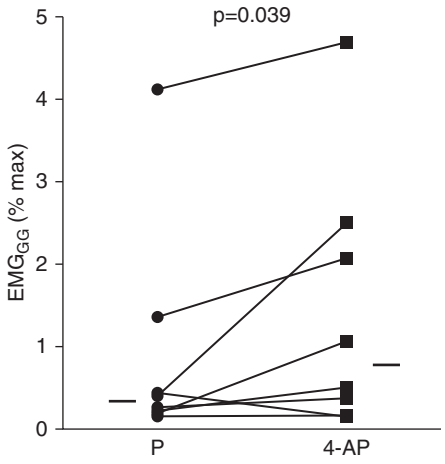

Figure 3. Individual data showing a significant increase of tonic genioglossus activity (EMG_{GG}) on 4-aminopyridine (4-AP) compared with placebo in eight participants in whom REM sleep was recorded on both study nights. This finding was limited to EMG_{GG} expressed as percentage of maximal voluntary activity ($\%_{max}$, see Table 2 and text for explanation). Horizontal lines indicate median values.

on 4-AP; $P > 0.5$) There was no difference in awake $\%_{max}$ EMG_{GG} activity before and after 4-AP administration for both tonic (0.32 [0.89] vs. 0.73 [0.97]; $P > 0.5$) and phasic activity (0.24 [0.98] vs. 0.51 [0.66]; $P > 0.5$). There was also no difference in EMG_{GG} activity, expressed as a percentage of baseline wakefulness, between placebo and 4-AP nights 3 hours after placebo/drug administration (tonic: 110 [42] on placebo vs. 97 [150] $\%_{wake}$ on 4-AP [$P > 0.5$]; phasic: 114 [70] on placebo versus 103 [99] $\%_{wake}$ on 4-AP; $P > 0.5$).

Effect of 4-AP on EMG_{GG} Activity during Sleep

The number of breaths analyzed during NREM sleep (off CPAP) was 1,006 ± 449 during placebo nights and 855 ± 452 during 4-AP nights. REM sleep off CPAP was present on both nights in eight of the 10 subjects, and the number of breaths analyzed was 385 ± 219 for placebo nights and 304 ± 203 for 4-AP nights.

Group data comparing EMG_{GG} expressed as μV, $\%_{max}$, and $\%_{wake}$ during sleep between nights are presented in Table 2 and Figure 2, respectively. There was no difference in tonic or phasic EMG_{GG} activity during NREM sleep between placebo and 4-AP nights. Tonic EMG_{GG} activity was higher during REM sleep on 4-AP compared with placebo, but only when expressed as $\%_{max}$ (Figure 3). Phasic EMG_{GG} activity during REM was not consistently altered by 4-AP (Table 2). EMG_{GG} values during REM sleep reflect both active periods characterized by sporadic muscle twitches and passive periods characterized by relative atonia, as 4-AP increased EMG_{GG} activity in both these periods in animal models (5).

Genioglossus Responsiveness

Genioglossus muscle responsiveness to progressively larger epiglottic pressure swings were unchanged between placebo and 4-AP nights as a group. However, seven of 10 participants had improvement in muscle responsiveness on 4-AP compared with placebo (Figure 4).

Effects on Upper Airway Collapsibility

Passive upper airway collapsibility was calculated in eight of 10 participants on both nights. One participant did not tolerate CPAP drops, and it was not possible to obtain flow-limited breaths during CPAP manipulation during placebo night in another participant. In the remaining eight participants, there was no significant difference in passive upper airway collapsibility between placebo and 4-AP nights (−7.6 [7.8] on placebo vs. −4.8 [5.0] cm H_2O on 4-AP; $P = 0.20$).

Effects on Sleep

4-AP had no significant effect on sleep efficiency or architecture. The arousal index was also similar between nights (Table 3). All subjects had an apnea–hypopnea index lower than 1 on both nights with the exception of subject 8, who had an apnea–hypopnea index of 7 on placebo versus one event/hour on 4-AP.

Discussion

The main finding of this study was that 4-AP increased EMG_{GG} tonic activity during REM sleep. However, the increase in genioglossus activity was mild and reached the statistical significance only when measured as a percentage of maximal voluntary activation, not when measured in μVs or as a percentage of quiet wakefulness. Compared with placebo, genioglossus activity on 4-AP was unchanged during

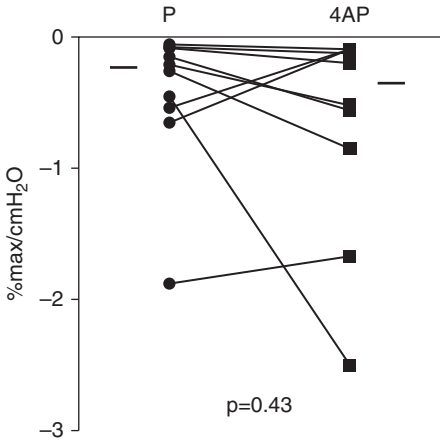

Figure 4. Individual data showing genioglossus muscle responsiveness on placebo (P) and 4-aminopyridine (4-AP). Although there was no statistically significant effect of 4-AP as a group, seven of 10 subjects had an improvement in genioglossus muscle responsiveness to progressively greater epiglottic pressure swings evaluated during slow continuous positive airway pressure dial down. However, with the exception of one participant, the improvement was minimal.

wakefulness or NREM sleep. Genioglossus muscle responsiveness to progressively greater epiglottic pressure swings and upper airway collapsibility during sleep were also unchanged between nights.

To our knowledge, this is the first time the effect of a K^+ channel blocker on genioglossus activity in humans has been tested. Previous research performed *in vitro* in rat respiratory muscles showed that administration of 4-AP and tetraethylammonium increased the twitch force of sternohyoid and diaphragm muscles (16). Topical administration of a K^+ channel blocker named AVE0118 in pigs upper airways showed a significant reduction in upper airway collapsibility mediated by increased genioglossus muscle activity during drug-induced sleep (17). More recently, studies in freely behaving rats have confirmed that blockade of potassium channels with several agents (barium, 4-AP, tetraethylammonium) constitutes an efficient strategy to increase genioglossus activity and reverse NREM hypotonia and REM atonia during sleep (5). Previous studies of 4-AP in humans revealed this drug was capable of reversing opioid-related respiratory depression in the postoperative setting (18, 19). Furthermore, it is currently prescribed to improve motor functions in people suffering from multiple sclerosis (8),

Table 3. Sleep parameters

	Placebo	4-AP	*P* value
TST, min	311.5 [65.8]	282.5 [55.0]	0.32
SE, %	84.9 [16.4]	79.3 [18.8]	0.38
NREM1, %TST	10.5 [5.9]	10.3 [13.5]	0.27
NREM2, %TST	50.3 [12.0]	54.7 [8.8]	0.32
NREM3, %TST	21.4 [8.1]	15.1 [5.8]	0.23
REM, %TST	16.7 [5.9]	12.1 [12.0]	0.13
AHI, events/hour	0.0 [0; 0]	0.0 [0; 0]	>0.5
ArI, events/hour	10.3 [6.8]	6.3 [7.5]	>0.5

Definition of abbreviations: 4-AP = 4-aminopyridine; AHI = apnea-hypopnea index; ArI = arousal index; NREM1, NREM2, and NREM3 = non-rapid eye movement sleep stages 1, 2, and 3; REM = rapid eye movement sleep; SE = sleep efficiency; TST = total sleep time.
Data are expressed as median [interquartile range].

an inflammatory disease of the central nervous system characterized by axonal demyelination. Indeed, it has been shown that in the presence of demyelinated neurons, blockade of K^+ channels leads to a dose-dependent increase in action potential amplitude and duration in animal models (20).

The most likely reason for the lack of an important effect of 4-AP on EMG_{GG} activity in our study is the small dose of the drug administered (10 mg by mouth). However, given its narrow therapeutic window, raising the 4-AP dose would be unsafe, as it would increase the risk for seizures and other severe adverse effects resulting from the wide distribution of voltage-gated K^+ channels in the human body, as demonstrated in clinical trials performed in patients with multiple sclerosis (21). The recent identification of a subfamily of potassium channels named G-coupled inwardly rectifying K^+ channels 2.4, whose distribution is largely limited to cranial motoneurons, opens the possibility of a more specific pharmacological target to be tested in future (5).

Limitations

Our study had several limitations. First, the study could be underpowered to establish the inefficacy of 4-AP on genioglossus activity, especially for the genioglossus muscle responsiveness to progressively greater epiglottic pressure swings, given that seven of 10 participants had an increase in genioglossus activity when receiving the drug compared with placebo. However, the mean difference was small ($-0.23 \pm 0.73\%_{max}$/cm H_2O), and to detect this difference with statistical significance, we would need to study 81 subjects, which is not feasible considering the techniques used. Second, the increase in REM EMG_{GG} tonic activity needs to be interpreted with caution: the raw EMG amplitude may vary with the electrodes and their site of insertion. For this reason, it is a common practice to normalize the raw data (μV) by the maximal EMG_{GG} activity during wakefulness or by the median wakefulness values (3, 22) collected over several minutes' recordings and without movement artifacts. Despite this, even the normalization procedures can be problematic as the maximum maneuvers are effort-dependent and may vary between nights, and wakefulness values can be affected by behavioral components that may be different from night to night.

Nevertheless, we found that wakefulness values and maximal maneuvers were very similar on and off the drug, suggesting 4-AP has no substantial effect on wakefulness and NREM EMG_{GG}, but may have some minor effect on tonic REM activity. However, given that this finding is limited to EMG_{GG} when expressed as a percentage of the maximum activation, it needs to be confirmed with further investigation. Third, we administered 4-AP only for 1 night, and we cannot exclude that a prolonged administration at the recommended dose of 10 mg twice daily could lead to different results, given that it was shown that 15 days of continuous administration of 4-AP 7.5 mg extended release twice a day increased the plasma concentration of 4-AP compared with the single dose in a group of healthy volunteers (23). As the main outcome of this study was to determine the effect of 4-AP on genioglossus muscle, we decided to study healthy volunteers, as their sleep is characterized by long periods of stable breathing in every sleep state. These periods allow a more reliable measurement of EMG_{GG} compared with the highly variable breathing pattern of patients with obstructive sleep apnea, in whom sleep is also frequently fragmented by arousals. However, given the difference in upper airway anatomy and physiology between patients with obstructive sleep apnea and normal patients (24), it may be possible that a study performed in patients with obstructive sleep apnea leads to different conclusions

Conclusions

In this study, we assessed the effect of the potassium channels blocker 4-AP on genioglossus activity and showed that it may have a small effect on tonic genioglossus activity in REM. Despite a strong rationale, blockade of K^+ channels with 4-AP is not likely a feasible strategy to improve upper airway dilator muscle activity during sleep, possibly because of the low dose of 4-AP that can be safely administered in humans. Future research should be oriented toward an antagonist of a K^+ channel whose expression is limited to the hypoglossal motor nucleus, or at least a limited number of cell types to reduce the adverse effects related to this pharmaceutical class. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

Acknowledgment: The authors thank Lauren Hess, Ronnie Lo, and Eric Siletzky for assistance in data collection and subjects' recruitment.

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